Thrombosis Research 128 (2011) 565–569

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Evaluation of a new set of automated chemiluminescense assays for anticardiolipin and anti-beta2-glycoprotein I antibodies in the laboratory diagnosis of the antiphospholipid syndrome

Lies Persijn, An-Sofie Decavele, Sofie Schouwers, Katrien Devreese *

Coagulation Laboratory, Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Ghent, Belgium

ARTICLE INFO

Article history:
Received 4 February 2011
Received in revised form 29 March 2011
Accepted 2 April 2011
Available online 6 May 2011

Keywords:
Antiphospholipid syndrome
anticardiolipin antibodies,
anti-beta2 glycoprotein I antibodies,
lupus anticoagulant, thrombosis,
Zenit RA analyzer, ELISA

ABSTRACT

Introduction: The laboratory diagnosis of antiphospholipid syndrome (APS) requires the demonstration of antiphospholipid antibodies (aPL): lupus anticoagulant (LAC) measured through coagulation assays, anticardiolipin IgG or IgM antibodies (aCL) and/or anti-β2glycoprotein I IgG or IgM antibodies (aβ2GPI), usually detected by ELISA

Materials and methods: We evaluated the diagnostic value of aCL and aβ2GPI measured by a new automated system using the chemiluminescence principle, the immunoanalyzer Zenit RA (Menarini).

Results: Results of aCL and aβ2GPI were correlated with the clinical background of the patients and with results of ELISA (n = 314). Correlated to the clinical background sensitivity/specificity ranged for aCL IgG between 7.5-45.2% / 54.2-98.8%, for aCL IgM 3.4-5.5% / 89.9-94%, for aβ2GPI IgG 5.5-25.3% / 75.6-100% and aβ2GPI IgM 3.4-4.8% / 89.9-92.3%, depending on the cut-off used. Sensitivity with manufacturer's cut-offs was comparable to ELISA, except for aβ2GPI IgG with a significantly lower sensitivity compared to ELISA (5.5% vs 11.6%).

In the APS patient population (n = 30) sensitivity of aCL IgG and aβ2GPI IgG was higher measured by ELISA compared to Zenit RA (46.7% *vs* 30.0%, and 46.7% *vs* 26.7%, respectively).

Agreement between Zenit RA results and ELISA results for the four parameters was moderate (Kappa-values ranging 0.509-0.565). Sensitivity was 38.5%, 53.3%, 40% and 69.2% for aCL IgG, aCL IgM, aβ2GPI IgG and aβ2GPI IgM, respectively, applying the highest cut-off value for Zenit RA, raising towards 64.3%, 100%, 57.1%, for aCL IgG, aCL IgM, aβ2GPI IgG, respectively, in a APS patient population.

Conclusions: The new technology of chemiluminescense for measuring aPL showed good performance characteristics. Interpretation of results with a cut-off value associated with a good discrimination for disease, resulted in a lower sensitivity for the diagnosis of APS for aβ2GPI IgG measured by Zenit RA assays compared to ELISA; sensitivity for aCL IgG was comparable to ELISA. Specificity for all parameters was high and comparable for aCL and aβ2GPI.

Introduction

The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the presence of antiphospholipid antibodies (aPL) and clinical symptoms categorized as thrombotic complications and pregnancy morbidity [1].

The laboratory diagnosis requires the demonstration of aPL: the lupus anticoagulant (LAC), anticardiolipin (aCL), or anti-beta2glycoprotein I (aβ2GPI) antibodies. LAC is identified by phospholipid-dependent coagulation tests detecting aPL as inhibitors of coagulation; aCL and aβ2GPI antibodies are demonstrated via an immunosorbent method, most commonly used is the enzyme-linked immunosorbent assay (ELISA). The revised Sapporo laboratory criteria (Sydney 2006) for APS include: the presence of LAC; the presence of aCL IgG and/or IgM isotype, present in medium or high titer (> 40 GPL or MPL, or>99th percentile) and the presence of $a\beta_2$GPI IgG and/or IgM (titer>99th percentile). Positive laboratory results have to be confirmed on two or more occasions, at least 12 weeks apart [1,2].

All assays for aPL, coagulation assays as well as ELISAs show methodological shortcomings [3], and therefore the search for better assays goes on. The assays for the detection of aPL antibodies must be sufficiently sensitive to classify patients correctly as APS positive. They also need to be highly specific since false positive results may have an impact on clinical decisions. Patients with thrombosis and aPL antibodies may be given indefinite oral anticoagulant treatment. Falsely diagnosed patients may thus be exposed to a high risk of bleeding, without having any benefit of such treatment. False negative results have serious consequences for patients suspected for APS

* Corresponding author. Tel.: +32 9 332 65 67; fax: +32 332 49 85.
E-mail address: katrien.devreese@uzgent.be (K. Devreese).

0049-3848/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2011.04.004

because they need long-term anticoagulation to prevent recurrences [4]. Despite the guidelines for measuring aCL and aβ2GPI proposed by the standardization group of the European Forum on Antiphospholipid antibodies [5–7] the laboratory diagnosis of the APS remains a challenge for each laboratory worker in the field. Automatization can improve the reproducibility and reduce interlaboratory variation, still a major problem in analysis of aCL and aβ2GPI antibodies [3].

The aim of our study was to evaluate the diagnostic value of aPL measured by a new automated system, the immunoanalyzer Zenit RA (A. Menarini diagnostics, Firenze, Italy).

Performance characteristics of the assays were evaluated and results of all assays were compared with an ELISA and correlated with the clinical criteria for APS. The diagnosis of APS is complicated by the lack of a golden standard. Our current practice (ELISA) cannot be considered as a golden standard for newer tests, therefore, antibody positivity detected by the Zenit RA was evaluated also against the clinical background of the patients.

Materials and methods

Sample preparation

Blood was drawn in 3.2% (0.109 mol/l) sodium citrate tubes (one part sodium citrate to nine parts venous blood). Platelet-poor plasma was prepared by double centrifugation at 2360 g for 15 minutes at room temperature. After LAC analysis, all patient samples were kept at −80 °C for further analysis. Permission was given by the Ethical Committee of the Ghent University Hospital to store patient plasmas for further analysis. Plasma collected from healthy volunteers, in order to calculate in-house cut-off values, was collected in the same way as the plasma collected from the patients.

Patient population

314 consecutive patient plasmas were selected from individuals referred to our thrombophilia centre or referred for autoimmune disease testing with request for LAC screening.

75/314 (23.9%) samples were classified as LAC-positive and 239/314 samples were LAC-negative.

The clinical features of APS (thrombotic episodes or pregnancy morbidity) were retrospectively identified by consulting the medical records: 146 fulfilled the clinical criteria for APS, 168 patients did not. 127/314 suffered from thrombosis, 19/314 had pregnancy complications. Part of the patients (n = 30) included in this study were true APS patients, defined by clinical criteria and at least one laboratory criterion persistently positive (LAC, aCL or aβ2GPI determined by ELISA).

Autoantibody assays

aCL and aβ2GPI antibodies were measured with ELISAs Bindazyme™ aCL and aβ2GPI S EIA kits (The Binding Site (BS), Birmingham, UK), the assay currently in use in our lab. These assays use, as an internal reference, the Koike monoclonal antibodies IgG HCAL and IgM EY2C9, chimeric monoclonal human IgG/IgM antibodies [8]. Results are expressed in μg/ml [9]. The cut-off value of the Bindazyme ELISAs was determined locally (99th percentile), on the same set of 50 healthy volunteers used for the in-house cut-off value calculation of the Zenit RA [9].

Zenit RA, a random-access immunoanalyzer, uses a two-step immunoassay method based on the principle of chemiluminescence. β2GPI or cardiolipin/β2GPI complex is used to coat magnetic particles (solid phase) and a human anti-IgG or anti-IgM is labeled with an acridine ester derivative (conjugate). The source of cardiolipin and β2GPI is bovine heart and human serum, respectively. During the first incubation, the specific antibodies present in the sample, in the calibrators, or in the controls bind with the solid phase. During the second incubation, the conjugate reacts with the antibodies captured on the solid phase. After each incubation, the material that has not bonded with the solid phase is removed by suction and repeated washing.

The quantity of marked conjugate bonded to the solid phase is evaluated by chemiluminescent reaction and measurement of the light signal. The generated signal, measured in RLU (Relative Light Units), is indicative of the concentration of the specific antibodies present in the sample, in the calibrators, and in the controls.

For aCL IgG or IgM the concentrations of the calibrators are expressed in GPL or MPL U/ml (U = units) and calibrated against the "Harris" reference sera. For aβ2GPI IgG or IgM the concentrations of the calibrators are expressed in AU/ml (Arbitrary Unit) and calibrated against an internal reference standard, not further specified by the manufacturer.

Every sample was analysed in duplicate (calibrators, controls, reference population and patient samples).

We determined in-house cut-off values, using 50 healthy normal individuals by the method of percentiles (99th) [6]. Quality control material, provided by the manufacturer, was analysed in every run. The APS IgM or IgG control set provides a ready-to-use positive control, containing a known quantity of aCL or aβ2GPI antibodies and a negative control containing normal human serum. Based on the results of the positive control imprecision characteristics were evaluated.

Lupus anticoagulant assays

LAC assays were performed according to the recommendations of the ISTH, using screening, mixing and confirmation tests and applying the updated guidelines [10,11] in an activated partial thromboplastin time (aPTT) and a diluted Russell's viper venom time (dRVVT) based test system.

Statistical analysis

Data were analysed using IBM SPSS Statistics 18 (PASW) (release 18.0.3, 2010). Sensitivity, specificity, positive and negative predictive values for each test were calculated against the clinical background of the patients, meaning that the presence of a clinical feature defines the patient as being 'ill'. Comparison of Zenit RA to ELISA was performed in the same way, being aware that our current practice (ELISA) is not the gold standard.

The Chi-square test is used to test the relationship between two classification systems (two-way classification between "ill-not ill" and positive-negative test result). If the calculated *P*-value (two-sided) is less than 0.05, there is a statistically significant relationship between the two classifications, i.e. the test result and the presence of a clinical feature. In this way the diagnostic power or the ability of a test to discriminate between patients with and without clinical criteria is reflected in the *P*-value.

Inter-rater agreement is used to evaluate the agreement between two classifications presented in a 2 × 2 frequency table; K is 1 when there is perfect agreement between the classification systems; K is 0 when there is no agreement better than chance.

A comparison of proportions (MedCalc® Version 7.1.0.0 (MedCalc Software, Mariakerke, Belgium) was performed to compare sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). A *P*-value equal or less than 0.05 was considered as statistically significantly different.

Results

Calculation of cut-off values

The cut-off values were calculated with the 99th percentile. Table 1 presents the calculated cut-off values of all Zenit assays in comparison with the reference values given by the manufacturer.